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(54) Title: PREBIOTICS AND PROBIOTICS

(57) Abstract

A method of enhancing a resident population of lactic acid-producing micro-organisms, preferably lactobacillus, in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganism and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of the resident population of lactic acid-producing microorganisms and the probiotic micro-organisms, if provided.

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## Prebiotics and probiotics

### Technical Field

This invention relates generally to the use of prebiotic and probiotic preparations to improve intestinal health and conditions related to microbial populations in the gastrointestinal tract of host humans and animals, and thereby improve the health and well being of the host.

### Background Art

The concept of probiotics was in use in the early 1900s, however, the term was only coined in 1965 and has subsequently evolved and numerous definitions have been proposed. Initially it was used to refer to the stimulation of the growth of one microbe by another i.e. the opposite of antibiotic. Today it is generally agreed that a probiotic is a preparation "of live microorganisms which, applied to human or animal, beneficially affects the host by improving the properties of the indigenous microbiota". Lactic acid bacteria and particularly lactobacilli are frequently used as probiotics. Bifidobacteria are also extensively used as probiotics. Furthermore, microbes of gastrointestinal origin or strains related to such microbes may also be used as probiotics.

The gastrointestinal (indigenous) microbiota contributes significantly to the health and well being of the host. It is a complex and diverse population which can reach up to  $10^{14}$  bacteria in an individual and may have both beneficial and harmful effects on the host. Some of the beneficial and harmful effects of the gastrointestinal microbiota are summarised in Table 1. It is envisaged that these parameters may be influenced by probiotic administration.

The gut microflora of an healthy subject protects the host from pathogen invasion, however, in the young, the elderly, and the compromised patient, this protective barrier is less effective. An individual can be compromised to various degrees, varying from minor stress related events to

preparations that may be potentially used to provide a carbohydrate source for desirable microbes.

TABLE 1  
Influences of the human intestinal microbiota on the host

Beneficial effects	Harmful effects
Inhibition of pathogens	Constipation
Stimulation of immune system	Diarrhoea
Synthesis of vitamins	Infections
Aid in digestion	Liver damage
Produce metabolic fuel for enterocytes	Cancer
Maintain stability of ecosystem	Flatulence
Metabolise drugs	Irritable bowel
	Ulcerative colitis
	Crohn's disease

Glucans are cell wall polysaccharides with glucose as the main component sugar having a beta-bonding with mostly beta 1,3 links and also beta 1,6 links. While originally isolated from black fungi, glucans have since been found in large quantities in yeast cell walls. Glucans have been shown to propagate indigenous bifidus bacteria in intestines and have been proposed to be useful as a pharmaceutical agent for conditioning the intestine. It has been reported that glucans can function as an immunostimulator (e.g. Brattgjerd *et al* 1994) when intraperitoneally administered.

Probiotic bacteria have been described to exert antimicrobial effects which refers to the actions of the probiotic preparation on another microbe or group of microbes. Some probiotic bacteria are directly applicable for enhancing resistance against intestinal pathogens, prevention of diarrhoea and constipation (reviewed by Fernandes *et al*; 1987; Katalaris, 1996; *Scandinavian Journal of Gastroenterology* 1996; 31: 100-105).

Microbial competition with the pathogen for the nutrients in the site of colonisation. As many gastrointestinal pathogens attach to the intestinal mucosa, the effects of probiotics on the ability of the pathogen to attach

components which inhibit attachment of enterotoxigenic *Escherichia coli* to intestinal mucosa (Blomberg *et al.*, 1993), however, there is no evidence as yet that this occurs in the digestive tract. In addition, various compounds produced during growth of the probiotic have been shown to inhibit 5 pathogen growth (reviewed by Fernandes *et al.*, 1987; Klaenhammer, 1988; Mishra and Lambert, 1996). These include organic acids such as lactic and acetic acid, reuterin and bacteriocins (Tagg *et al.*, 1976). The organic acids lower the pH and thereby can indirectly affect growth of the pathogen. In addition, the lactic and acetic acids produced by these organisms can be 10 toxic to microbes. Reuterin which inhibits the growth of a very broad range of cells (Lindgren & Dobrogosz, 1990), is produced by *Lactobacillus reuteri* when grown in the presence of glycerol. Numerous bacteriocins have been 15 reported to be produced by lactobacilli e.g. Acidophilin, Acidolin, Lactocidin, Bacteriocin, Bulgarican, Lactolin, Lactobacillin and Lactobrevin (reviewed by Fernandes *et al.*, 1987; Klaenhammer, 1988). Bacteriocins can either have a very broad range of activity or alternatively specifically inhibit the growth of a very limited range of closely related microbes. For example, *Lactobacillus* sp exhibited specific antagonistic effects towards *Clostridium ramosum* (McCormick & Savage, 1983).

20 The present inventors have surprisingly found that beta-glucans can be used to influence pathogen adhesion, alter microbial populations in the gastrointestinal tract, improve the effectiveness of probiotic compositions, and promote growth of a probiotic microorganism. A range of crude and pure preparations of beta-glucans have been shown to be effective.

25 Disclosure of the Invention

In a first aspect, the present invention consists in a method of enhancing a resident population of lactic acid-producing microorganisms in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof.

site in the gastrointestinal tract where it is utilized by a resident population of micro-organism and/or the introduced probiotic micro-organisms, if

resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided.

In a second aspect, the present invention consists in a method of suppressing an undesired population of microorganisms in a total microorganism population in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganism and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of a resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided, and suppressing the growth and/or activity of the undesired population of microorganism

In a preferred form, the undesired population of microorganisms is enteric pathogens. As will be appreciated, there are a large number of pathogens including Gram negative bacteria like *Salmonella*, *Escherichia coli*, *Campylobacter*, *Helicobacter*, *Vibrio*, and *Pseudomonas*; Gram positive bacteria like *Clostridium*; viruses like Norwalk virus, Norwalk-like viruses and Rotavirus; and protozoa like *Cryptosporidium*, *Entamoeba*, *Giardia*, and *Dientamoeba*. The present invention would be suitable for the suppression of many of these pathogens in the gastrointestinal tract of animals.

Suitable beta-glucans include those originating from (a) plants including cereals such as oats and barley, (b) fungi, (c) yeast, and (d) bacteria. In addition, microbial cell wall preparations and whole cells rich in beta-glucans are also suitable sources for beta-glucan preparations useful for the present invention. Monomer residues in glucans can have 1-3 and 1-4, or

1-6 linkages. Beta-glucans having 1-3 linkages are particularly preferred. *Agaricus* beta-glucans are particularly preferred for the present invention. It will be appreciated, however, that other beta-glucans would also be suitable. A

and/or probiotic microorganisms can be used. One percent has been found to be particularly suitable.

The resident lactic acid-producing microorganism is preferably a lactobacillus, and more preferably *Lactobacillus fermentum*.

5 In a preferred form, the present invention further includes one or more oligosaccharides, polysaccharides or other prebiotics. A concentration of oligosaccharides, polysaccharides or other prebiotics of about 0.001 g to 10 g per kg body weight per day is preferred.

10 Preferably, the probiotic microorganism is selected from the group consisting of lactic acid-producing microorganisms, bifidobacterium, yeast, and mixtures thereof. Preferably, the lactic acid-producing microorganism is a lactobacillus, more preferably *Lactobacillus fermentum*. Preferably, the yeast is a *Saccharomyces* sp.

15 Typical concentration range of probiotic microorganisms is  $10^3$  to  $10^{13}$  cells per day.

20 Although oligosaccharides have been shown to promote bifidobacteria numbers in animals, prior to the present invention, it was thought that lactobacillus numbers cannot be changed in a similar manner. Gibson et al 1995 found that oligofructose and inulin, when fed to humans, selectively stimulated the growth of bifidobacteria without influencing the numbers of lactobacillus. From these results and observations by others in the field, it would not be expected that beta-glucans would positively effect the growth and/or activity of lactic acid-producing microorganisms, particularly lactobacillus, *in vivo* as found by the present inventors.

25 Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Brief Description of Drawings

Figure 1 shows the results of *Lactobacillus* in the gastrointestinal tract of animals fed beta-glucan or control diet; and

5 Figure 2 shows faecal beta-glucanolytic microorganisms of animals fed beta-glucan or control diet. Analysis of beta-glucanolytic activity on agar plates containing beta-glucan from yeast cell sacs.

Modes for Carrying Out the Invention

## EXAMPLE 1

10 This has been studied by enumerating coliforms, bifidobacterium and lactobacilli *in vitro* in the presence of beta-glucan. The beta-glucan used in this example was prepared from yeast cell sacs. More specifically, aliquots (1 ml) of human faecal homogenates (10 g per 100 ml diluent) were added to diluted WC broth (diluted 50:50 with 0.05M phosphate buffer) to which were added the beta-glucan and a lactobacillus or bifidobacterium strain and 15 various combinations. For each of the combinations, parallel tubes were prepared with one set being inoculated with *Bifidobacterium* spp or *Lactobacillus* spp. All mixtures were then incubated for up to 24 hours and bacterial numbers enumerated. Results (Table 2) are expressed as the numbers of coliforms, total lactobacilli, total bifidobacterium, or total 20 anaerobes when enumerated as colony forming units. It can be seen that coliform numbers are unchanged or reduced, while numbers of bifidobacteria and lactobacilli can be increased up to 10-fold, and selected groups within a genus can be enhanced.

TABLE 2  
Effect of 1% beta-glucan on *Lactobacillus* and *Bifidobacterium*

Culture mix	Coliforms	Bacterial numbers (CFU/ml)			<i>Bifidobacterium</i>	
		<i>Lactobacillus</i>		Large Colony		
		Total	Large Colony			
Faecal homogenate (FH)	$3.2 \times 10^6$	$3.4 \times 10^5$	$1.2 \times 10^5$		<2	
FH + 1% glucan	$5.2 \times 10^6$	$5.3 \times 10^5$	$4.8 \times 10^5$		<2	
FH + <i>Bifidobacterium</i> (SUB)	NA	NA	NA		$1.0 \times 10^6$	
FH + SUB + 1% glucan	NA	NA	NA		$1.2 \times 10^7$	
FH + <i>L. fermentum</i> (Lf)	NA	$2.8 \times 10^5$	$2.8 \times 10^5$		NA	
FH + Lf + 1% glucan	NA	$1.2 \times 10^6$	$1.2 \times 10^6$		NA	

NA - not analysed

5

## EXAMPLE 2

This example demonstrates that dietary supplementation with beta-glucan stimulates gastrointestinal populations of *Lactobacillus*. In this study, 10 animals (3 per group) were provided either beta-glucan diet or control diet (Table 3). The beta-glucan used in this example was prepared from yeast cell 15 sacs. After 6 weeks feeding, animals were euthanised and luminal content collected from stomach, ileum, caecum and colon. Collected material was homogenised and diluted in Wilkens-Charlgren broth (Oxoid). Dilutions were spread on to Rogosa agar (Oxoid) and incubated anaerobically for 48 h.

15 Beta-glucan was prepared from yeast cell wall material.

100% of the material was composed of beta-1,3 and beta-1,6 linkages.

TABLE 3  
Composition of beta-glucan and sucrose diet

Ingredient	Amount (Glucan diet)	Amount (Control diet)
Beta-glucan	450	0
Sucrose	150	600
Casein	200	200
Sunflower	25	25
Canola oil	25	25
Gelatin	20	20
Wheat bran	100	100
Choline chloride	2	2
Methionine	3	3
Standard mineral mix	35	35
Standard vitamin mix	10	10

5 EXAMPLE 3

In addition to studying effects on microbial growth, effects on adhesion have been investigated. Desirable bacteria such as lactobacillus, as well as undesirable pathogenic *E. coli* and *Salmonella* sp have been grown either in a standard laboratory broth or in this broth supplemented with beta-glucans. The beta-glucan used in this example was prepared from yeast cell sacs. Bacterial suspensions were incubated with mucosal pieces from various regions of the gastrointestinal tract. These mucosal pieces were either used directly or after pre-treatment with the beta-glucan. Results are shown in Table 4. While adhesion of the pathogen was adversely affected, the adhesion of the lactobacillus was increased by the presence of the beta-glucan.

TABLE 4  
Effect of beta-glucans on adhesion

Bacteria	Bacteria adhering (per mg tissue)		
	Control tissue	Tissue + 1% glucan	Reduction (%)
<i>S. typhimurium</i>	$1.69 \times 10^8$	$5.53 \times 10^7$	~ 65
<i>E. coli</i> K88	$6.07 \times 10^7$	$3.43 \times 10^7$	~ 50
<i>L. fermentum</i>	$5.37 \times 10^8$	$6.2 \times 10^8$	0

## EXAMPLE 4

5 This example demonstrates that dietary supplementation with beta-glucan increases resistance to gastrointestinal infection by *Clostridium difficile* and *S. typhimurium* and that this effect is enhanced when glucan-treated mice were also orally dosed with *Saccharomyces* sp cell suspension. The beta-glucan used in this example was acid and alkali washed cell sacs of yeast. The *Saccharomyces* sp alone reduced numbers of *C. difficile* to some extent and markedly reduced *S. typhimurium* numbers. Groups of animals (4 groups, 8 animals per group) were provided either beta-glucan diet or control diet (Table 3). After feeding diets for four weeks, four groups were supplied drinking water containing cefoxitin (0.032 g/L) and D-cycloserine (1.0 g/L) and subsequently challenged with *C. difficile* ( $10^{10}$  CFU). Another four groups were supplied drinking water containing streptomycin (5 g/L) and neomycin (5 g/L). These animals were subsequently challenged with *S. typhimurium* ( $10^{10}$  CFU). After pathogen challenge, animals were oregastrically dosed with either *Saccharomyces* sp (strain FII 542900) ( $10^8$  CFU in YPG broth), or with fresh YPG broth. Development of infections were monitored daily by analysis of faecal *S. typhimurium* or *C. difficile*. Faecal material was homogenised and diluted in Wilkins-Charlton broth (Oxoid).

10 The results of this experiment are shown in Table 5. The faecal levels of *C. difficile* were reduced in animals fed beta-glucan, about 2.5 g units less than what was

15 observed in the control group. The faecal levels of *S. typhimurium* were reduced in animals fed beta-glucan, about 2.5 g units less than what was

glucan treated mice, while a log 3 reduction in mice dosed with *Saccharomyces sp* alone ( $P<0.05$ ). The combination of beta-glucan diet and *Saccharomyces sp* oral administration resulted in a significant reduction ( $P<0.05$ ) in faecal numbers of both pathogens when compared to both the 5 control diet and the *Saccharomyces sp* dosed mice (Table 5). This reduction corresponded to 3 and  $>4$  log units for *C. difficile* and *S. typhimurium*, respectively. This corresponded to a marked improvement in weight loss and other signs of severe infection in animals treated with the combination of 10 beta-glucan and *Saccharomyces sp*. It can be concluded that a beneficial synergistic effect can be achieved by beta-glucan alone and enhanced by the combinations of beta-glucan and *Saccharomyces sp*.

TABLE 5

Reduction of pathogens by oral administration of *Saccharomyces sp* to beta- 15 glucan fed mice that were challenged with either *C. difficile* or *S. typhimurium*

Diet	Reduction (log CFU per g faeces)	
	<i>C. difficile</i>	<i>S. typhimurium</i>
beta-glucan	1.5*	$\leq 1$
beta-glucan and <i>Saccharomyces sp</i>	3**	$>4**$
<i>Saccharomyces sp</i>	0.8	3*

\* Significantly different from beta-glucan free diet ( $P<0.05$ )

20 \*\* Significantly different from beta-glucan-free diet and *Saccharomyces sp* alone.

## EXAMPLE 5

This example demonstrates that:

beta-glucan can reduce the numbers of pathogenic microorganisms,

In this study, animals (8 per group) were provided either beta-glucan

or beta-glucan and *Saccharomyces sp* orally.

After 10 days, the animals were challenged with *C. difficile* and *S. typhimurium*.

Diluted material was spread on to beta-glucan agar (Table 6) and incubated anaerobically for 75 h. Clear zones developed around colonies formed by beta-glucanolytic microorganisms.

Relative to animals fed the control diet, density of beta-glucanolytic population was up to 100 fold greater in animals fed beta-glucan diet (Fig. 2).

TABLE 6  
Composition of beta-glucan agar

Ingredient	Amount (g/L)
Yeast extract	2.5
Trytone	5.0
Peptone	7.5
beta-glucan	10
Cysteine	0.5
NaCl	2.0
K <sub>2</sub> HPO <sub>4</sub>	2.0
KH <sub>2</sub> PO <sub>4</sub>	1.0
NaHCO <sub>3</sub>	2.0
MgCl <sub>2</sub>	0.2
CaCl <sub>2</sub>	0.2
CoCl <sub>2</sub>	0.02
MnCl <sub>2</sub>	0.02
FeSO <sub>4</sub>	0.005
Tween 80	2
Hemin	0.005
Vitamin B <sub>12</sub>	0.001
Vitamin K	0.0005
Agar	13.0

## EXAMPLE 6

This example demonstrates that a range of beta-glucans induce the observed effects. Beta-glucan prepared from a number of *Saccharomyces cerevisiae* strains and of bacterial origin were used. Both crude forms and more purified forms were tested. The crude forms were whole cell sacs that were washed 2-3 times in water and spray dried. The more purified forms were prepared from the water washed preparations that were subsequently alkali washed, then acid washed and then water washed prior to spray drying. When these various beta-glucan forms were incubated with faecal homogenates diluted in laboratory medium, all forms were degraded (Table 7) and were selectively utilised by lactobacilli when tested using lactobacillus pure cultures on glucan agar (Table 6).

15 TABLE 7  
Fermentation of beta-glucan preparations by mixed fecal slurries

	Beta-glucan sample				
	1	2	3	4	5
Degradation (%)	50	>60	>60	>70	>50
Cell sac per field:					
0 hour	156	NA	NA	clumped	NA
1/2 hours	9.2	NA	NA	75	NA
Dry residues (g)	0.3048	0.2630	0.3097	0.3950	NA
Total anaerobes	$2.9 \times 10^6$	$3.1 \times 10^7$	$1.4 \times 10^7$	$1.9 \times 10^7$	NA

Beta-glucan 1 - water-washed *Saccharomyces* strain A cell sacs

Beta-glucan 2

Beta-glucan 4 - water-washed *Saccharomyces* strain B cell sacs

## EXAMPLE 7

This example demonstrates that yeast sac beta-glucan is poorly degraded in the upper regions of the mouse digestive tract and that most of the degradation occurs in the caecum and colon of the animal where the microbial population is greatest. Two groups of three SPF Balb/c mice were fed for 60 hours with a diet containing 40% w/w yeast cell sac beta-glucan. After the mice were sacrificed, the stomach, duodenum, ileum, jejunum, caecum and faeces were sampled. Gastrointestinal contents were squeezed from the gut sections and faecal samples were individually homogenised with 50 mM phosphate buffer and a drop of the homogenate was placed on microscope slides. The degradation of the beta-glucan from mouse intestinal tract samples was examined using phase-contrast microscopy. The beta-glucan granules were degraded by less than 20% in the stomach and small intestine while greater than 70% degradation was noted in the caecum and faecal homogenates (Table 8).

TABLE 8  
Degradation of beta-glucan in mouse intestinal tract

	Sample source					
	Stomach	Duodenum	Ilium	Jejunum	Caecum	Faeces
Degradation	<10%	NO	<20%	>20%	>70%	>80%

20

NO - Not Observed: Few yeast cell sacs were observed in that area, probably because of the rapid transit time.

## USES

5 The present invention can be applied to all conditions in which microbes are identified or proposed as causative agents of disease in both human and animals and which can be advantageously effected by altering the numbers and/or activities of beneficial microflora of the gastrointestinal tract.

10 As infective diarrhoea has been shown to be improved by probiotic dosage, the present invention can be used to enhance the effect of the probiotic by itself and to reduce the microbial-induced diarrhoea when orally dosed alone. In addition, the present invention may be used effectively to 15 improve non-infective diarrhoea which has not been shown to be influenced by probiotics alone. It could be effective in reducing the effects of dietary related diarrhoea problems.

15 Infective diarrhoea refers to all cases of diarrhoea, both acute and chronic, in which the causative agents can be shown to be microbial, including bacterial, viral and protozoan. Such infective diarrhoea can manifest itself in a number of ways e.g. (a) infantile and geriatric diarrhoea; (b) antibiotic associated diarrhoea; (c) traveller's diarrhoea; (d) stressed-induced diarrhoea.

20 Both prophylactic and therapeutic uses are described in the present specification. The former can relate to prevention when the individual can be exposed to potential problems e.g. (i) investigative gastrointestinal examination when the bowel is decontaminated and can then be recolonised by an undesirable microbial population; (ii) travellers exposed to an altered 25 pathogen load or an alteration of the gut ecosystem because of various factors including diet change, which can predispose the individual to a lower infective dose of a pathogen. Therapeutic uses relate to the treatment of established conditions related to an undesirable balance of the gut microflora or an established pathogen infection.

1. As a general gut microflora stabiliser

ii. In clinical conditions directly or indirectly related to gastrointestinal

disorders, including but not limited to, diarrhoea, constipation, indigestion, flatulence, bloating, abdominal pain, and/or cramps.

sleep disorders, rheumatoid arthritis, chronic fatigue syndrome, hyperactivity, and ulcerative colitis

- 5       iii.     improved intestinal health
- iv.     directly influence the metabolism of the microbes such that the beneficial activities are enhanced e.g. elevated levels of antimicrobials or butyrate, and the harmful activities are reduced. e.g. reduced toxin production
- 10      v.     directly influence numbers of desirable microbes either directly or indirectly in the complex mix of gastrointestinal microbes
- vi.     directly influence numbers of undesirable microbes either directly or indirectly in the gastrointestinal system.

15       The present inventors have found that beta-glucans and oligosaccharides are carbohydrates which can be selectively used by beneficial microbes in the gut which in turn can suppress the numbers of the undesirable microbes.

20       It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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## CLAIMS:

1. A method of enhancing a resident population of lactic acid-producing microorganisms in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganisms and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of the resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided.
2. A method of suppressing an undesired population of microorganisms in a total microorganism population in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganism and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of a resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided, and suppressing the growth and/or activity of the undesired population of microorganism.
3. The method according to claim 2 wherein the undesired population of microorganism is an enteric pathogen.
4. The method according to claim 1, 2 or 3 wherein the beta-glucan is a preparation derived from plants, fungi, yeast, or bacteria.

6. A method of

7. The method according to claim 6 wherein the *Saccharomyces* is

8. The method according to any one of claims 1 to 7 wherein the beta-glucan is used at a concentration of up to 10% (w/w) when combined with other prebiotics and/or probiotic microorganisms.
9. The method according to claim 8 wherein the beta-glucan is used at a concentration of 1% (w/w).
10. The method according to any one of claims 1 to 9 wherein the resident lactic acid-producing microorganisms are *lactobacillus*.
11. The method according to claim 10 wherein the *lactobacillus* is *Lactobacillus fermentum*.
12. The method according to any one of claims 1 to 11 wherein the probiotic microorganisms are selected from the group consisting of lactic acid-producing microorganisms, *bifidobacterium*, yeast, and mixtures thereof.
13. The method according to claim 12 wherein the lactic acid-producing microorganisms are *lactobacillus*.
14. The method according to claim 13 wherein the *lactobacillus* is *Lactobacillus fermentum*.
15. The method according to claim 12 wherein the yeast is a *Saccharomyces sp.*
16. The method according to any one of claims 12 to 15 wherein the probiotic microorganism is administered at a concentration of  $10^3$  to  $10^{13}$  cells per day.

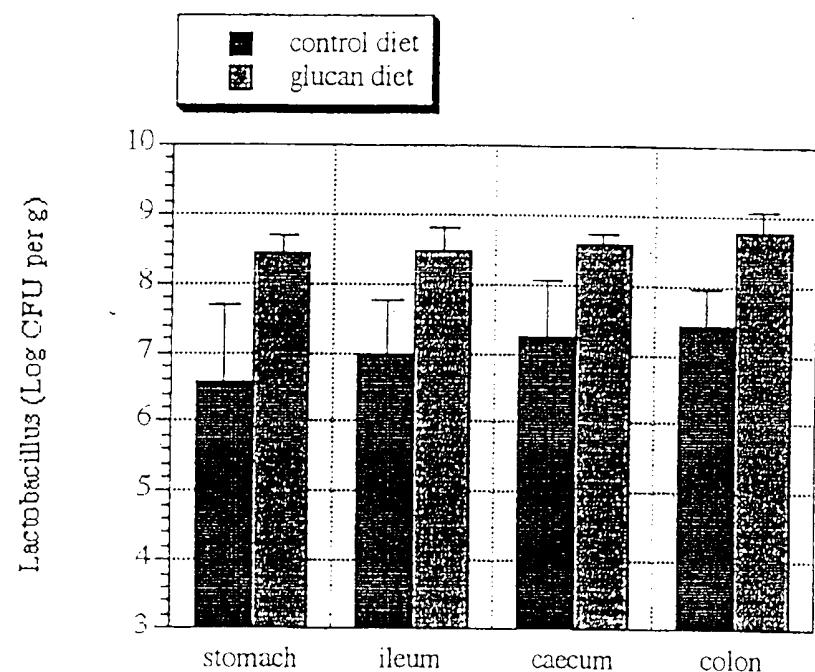
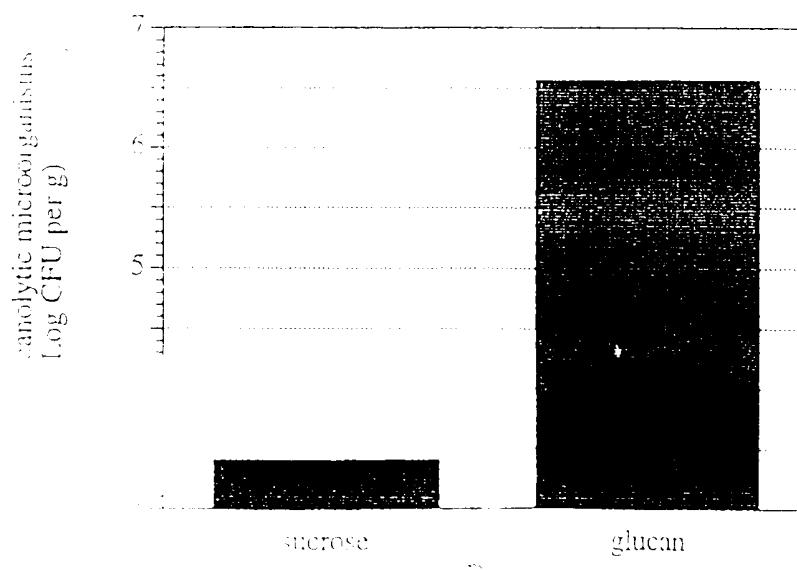


Fig 1



INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/AU 97/00859

#### A. CLASSIFICATION OF SUBJECT MATTER

Int Cl<sup>6</sup>: A61K 031/715

According to International Patent Classification (IPC) or to both national classification and IPC

#### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols):

A61K 031/715

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
DERWENT DATABASE, glucan, yeast, *saccharomyces*, bowel, diarrhoea

STN INTERNATIONAL KEYWORDS: glucan, yeast, *Saccharomyces cerevisiae*, bowel, diarrhoea, constipation, colon, sleep, arthritis, chronic fatigue syndrome, colitis, gastrointestinal, enteric, flora, microflora, gut, intestine, lactobacteria

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Patent Abstracts of Japan JP 08157377 A (NIPPON OIL CO LTD) 18 June 1996 - abstract	I-16
X	US 5576015 A (B A DONZIS) 19 November 1996 see whole document	I-16
P,X	AU 61824/96 A (CARLTON AND UNITED BREWERIES LIMITED) 23 January 1997 see whole document	I-16
X	WO 96/26732 A (NEWPHARMA Srl) 6 September 1996 see whole document	I-16

Further documents are listed in the continuation of Box C

X See patent family annex

#### Special categories of cited documents:

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone.

10 MAR 1995

BERNARD NIET

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00859

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU 97/00859
Category*	Relevant to claim No.	
X	WO 90/04334 A (ALPHA BETA TECHNOLOGY) 3 May 1990 see whole document	1-16
P,X	Front Foods Food Ingredients (1997), 2 (New Technologies for Healthy Foods & Nutraceuticals), pages 53-70. Manssur Yalpani, "Nutraceuticals - A Materials-Based Perspective" see in particular pages 63-65	1-16

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/AU 97/00859

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
AU	61824/96	WO	97/0123				
WO	90/04334	CA	900428	AU	44924/89	DE	940707
		EP	910814	JP	921022	NZ	970526
		US	901009	ZA	911030		

SEARCHER'S SIGNATURE